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Quantitation of latex allergens

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Abstract

Minimizing allergen concentration in latex goods to prevent sensitization to natural rubber latex (NRL) and thereby the development of clinical allergy is acknowledged as of mutual interest for rubber manufacturers and regulatory health authorities. However, measuring total protein, the principal currently available method, cannot be deemed a satisfactory regulatory measure to control allergen content. Specific methods based on human IgE-containing reagents, such as radioallergosorbent test (RAST) inhibition, have been available in certain laboratories for demonstrating NRL allergens in rubber products but the methods lack standardization. Currently, one commercial test has become available for measuring individual NRL allergens by capture ELISA-based assays using monoclonal antibodies and purified or recombinant allergens. Such methods are specific, they can be properly standardized, and they are of sufficient sensitivity and reproducibility. Results from medical gloves collected in two national market surveys in Finland in 1995 and 1999, respectively, show that Hev b 6.02 and Hev b 5, the two major allergens for NRL-allergic adults, are the most abundant allergens regularly detectable in high- and moderate-allergen gloves. In addition, Hev b 3 and Hev b 1, the two major allergens for children with spina bifida, are also commonly found. In general, when the sum of the four allergens exceeded 1 μg/g, most NRL-allergic patients showed positive skin prick test reactions against them. Using these new methods assessment of threshold levels that could in due course become guidelines for the rubber industry and regulatory health authorities is becoming possible. Eventually, this progress is expected to lead to a declining incidence of latex allergy.

Keywords: Latex allergy; Allergens; IgE; Capture enzyme-linked immunosorbent assay; Monoclonal antibodies; Recombinant proteins; Medical gloves

1. Introduction

Allergy to natural rubber latex (NRL) proteins has been known for about 20 years and is currently a well-recognized health problem among subjects using protective gloves and/or otherwise exposed to products made of NRL. A major group at risk of immediate hypersensitivity reactions to NRL consists of health care workers among whom the prevalence of latex allergy has been reported to be between 3 and 17% [1]. Importantly, NRL gloves are also the major contributors to latex aeroallergen levels in operating rooms [2]. Several studies have documented considerable differences between the allergen content of latex gloves made by different manufacturers, and even between gloves of different batches from a single manufacturer [3–5]. Other manufactured NRL products, such as household gloves, catheters, condoms, baby pacifiers, and toy balloons may also contain allergenic proteins [1].

There has been a growing need to monitor the allergenicity of gloves and other latex goods to prevent sensitization and clinical allergy but the availability of specific methods has been scanty. Total protein measurement has been shown to correlate relatively well with the true allergen contents measured by skin prick test (SPT) or human IgE-based immunologic inhibition assays [3–6]. Yet, such methods measure also nonallergenic proteins that are likely to be irrelevant in assessing allergenicity of the product. Therefore, allergen-specific assays are anticipated to provide much more reliable information. Unfortunately, however, most methods

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used for this purpose are based on the use of human IgE-containing sera and standards that still lack adequate validation and are not easily available. As an interim solution to the problem, regulatory health authorities in the United States (Food and Drug Administration; FDA) and Europe (European Committee for Standardisation, CEN) have advocated the measurement of extractable total protein as a surrogate marker for glove manufacturers to monitor their products.

Recently, substantial progress has been made in the development of specific and quantitative assays for individual latex allergens. When properly evaluated and validated such methods are likely to provide adequate means for measuring the allergen content of NRL products. In this paper currently available specific methods for measuring and quantitating NRL allergens are reviewed. Emphasis is given to a recently developed capture-ELISA method which is based on the use of monoclonal and/or polyclonal antibodies to a selection of clinically relevant NRL allergens that have been purified or produced by recombinant DNA technology.

1.1. NRL allergens in the source material and in manufactured products

Of the more than 200 different proteins or polypeptides in the liquid latex of the rubber tree, *Hevea brasiliensis*, only about one-fourth are allergens, meaning that they can initiate IgE antibody formation [7]. Substantial progress has been made in the purification and molecular characterization of NRL allergens, which has facilitated the assessment of their significance. The WHO/IUIS Allergen Nomenclature Committee (www.allergen.org) now lists 11 NRL allergens characterized at the molecular level. Most of these allergens have been cloned and produced by recombinant DNA techniques. Some of these allergens can be found in manufactured products and should therefore be considered to have clinical relevance in NRL allergy. It is also possible that new allergenic epitopes are formed in some proteins or peptides during glove manufacturing. Evidence to support this possibility was provided by Mäkinen-Kiljunen et al. [8], who demonstrated by immunoelectrophoresis that one allergen was present only in the glove extract but not in NRL.

It is generally agreed that both the protein content and allergen content can vary considerably in NRL gloves. Twenty- to one hundred-fold differences have been demonstrated in protein concentrations, and up to 3000-fold differences in the allergen contents, of various NRL glove brands [4]. Several studies have indicated that the total protein content measured by the modified Lowry method often correlates relatively well with the true allergen content but discrepant findings are not uncommon. Some NRL glove brands have been described with rather high total protein content but with low NRL allergen concentration and *vice versa*. Other proteins, such as casein, may also be added to the NRL during glove manufacture, which then can increase the total protein content of the glove. Therefore, at the level of individual gloves, protein concentration is neither specific nor accurate enough to draw conclusions on the safety of NRL gloves.

Knowledge about the presence of specific NRL allergens in NRL gloves and other manufactured rubber products is rapidly increasing but still scanty. There is at present convincing evidence showing that some allergens or immunologically active fragments of them can resist the harsh rubber manufacturing process and be demonstrated in the end products as IgE-binding motifs. The majority of the IgE binding capacity of one highly allergenic glove was attributable in a recent study to Heb 6.02, hevein [9]. Both Hev b 1 (rubber elongation factor or REF) and Hev b 3, (a 22–27-kDa rubber particle-associated protein), as well as the 16-kDa acidic protein (Hev b 5), have been identified in certain glove brands [10–12]. Evidence has also been presented that Hev b 2 (36-kDa rubber tree gluconase) can be present in latex glove extracts [13].

2. Description of methods

2.1. Methods available to measure NRL allergen activity

2.1.1. Methods based on immunoelectrophoresis and immunoblotting

Numerous studies based on immunoelectrophoretic methods and/or immunoblotting have described a large variety of NRL proteins binding IgE from sera of NRL-allergic patients but it is agreed that these methods carry marked limitations and are not suitable for quantitation of allergens. For example, as many as 26 IgE-binding polypeptides with molecular weights of about 14 kDa and isoelectric points ranging from pH 4.2 to 6.5 could be detected in NRL, indicating that the 14-kDa band is composed of a multitude of IgE-binding polypeptides with various isoelectric points [7].

2.1.2. Skin prick testing in voluntary latex-allergic subjects

Turjanmaa et al. [3] were the first to show that extracts of latex gloves can cause positive skin prick test (SPT) reactions in patients allergic to NRL. Allergenicity can be assessed by SPT in a semiquantitative manner since the size of the reaction is dependent on and directly proportional to the quantity of the allergens to which the patient has IgE class antibodies. From the biological point of view SPT would make an ideal test, but due to, e.g., ethical constraints, this approach cannot be routinely used as test for monitoring allergen content in latex gloves.

2.1.3. Human IgE-based immunologic inhibition assays measuring "total" allergen content

Two types of assays are available both based on the same (inhibition) principle: RAST inhibition and ELISA inhibition (RAST, radioallergosorbent test; ELISA, enzyme-linked immunosorbent assay). The critical reagent is pooled human serum containing IgE antibodies to relevant NRL allergens. The binding of these antibodies to NRL proteins immobilized onto a proper solid phase, such as a micotiter plate, is inhibited by serial dilutions of extracts of gloves or other NRL products. As a standard either native (nonammoniated) NRL or ammoniated NRL can be used. In studies performed by our group, the unammoniated source material, liquid NRL, has been assigned an arbitrary concentration of 100,000 allergen units (AU)/ml. The decrease in binding of IgE from the serum pool to the solid-phase antigens (allergens) is directly proportional to the concentration of allergens in the extract tested. These tests belong to the first methods used to provide reliable information on allergen content of latex gloves [4,5,14]. Highly significant correlations ($r = 0.94$–0.96) have emerged between the results of RAST inhibition and ELISA inhibition and the results of SPT, the "gold standard" for diagnosing latex allergy [5]. However, it has not been possible to properly standardize these assays, because of the lack of availability of standardized human IgE antibodies and/or of standardized allergens. One particular drawback is the scarcity of human sera containing IgE antibodies to Hev b 1 and Hev b 3.

2.2. Quantitation of individual allergens

2.2.1. Hev b 1 assay

Hev b 1 was originally extracted from NRL gloves [10]. Recently, a two-site monoclonal antibody-based assay has been developed to measure Hev b 1 levels in NRL products [15]. In this study with five different brands of NRL gloves, Hev b 1 concentrations were found to be in the range 18–40 μg/g rubber material, corresponding to 2–4% of the total extractable protein content in NRL glove extracts.

2.2.2. Performance of a capture-EIA kit developed for quantitation of four clinically relevant NRL allergens: Hev b 1, >Hev b 3, Hev b 5, and Hev b 6.02

2.2.2.1. Properties of the allergen determination kit. Hev b 6.02 and Hev b 5 are the two most important latex allergens for health care workers, while Hev b 1 and Hev

Table 1
Concentrations of four latex allergens in 22 glove extracts as compared with SPT reactivity, total allergen level, and protein assays[a]

Glove No.	Allergen concentration in glove extracts							SPT reactivity		
	Hev b 1[b]	Hev b 3[b]	Hev b 5[b]	Hev b 6.02[b]	Allergen sum (μg/g)	Modfied Lowry (μg/g)	IgE-EL-ISA-I[c]	SPT-histamine ratio[d]	No. of reactive patients	Percent positive patients
3	0	0	0	0.03	0.03	23	1.1	0.18	2	10
1	0	0	0	0	0.00	45	1.1	0.00	0	0
2	0	0	0	0	0.00	65	2.1	0.27	6	30
4	0	0.22	0	0.02	0.24	117	2.3	0.2	4	20
12	0	0	0.03	0.35	0.38	620	2.4	0.46	10	50
5	0	0	0	0	0.00	99	2.8	0.00	2	10
8	0	0	0.03	0.05	0.07	112	2.8	0.31	5	20
10	0	0	0.05	0.9	0.99	292	3.8	0.4	6	30
6	0	0	0.03	0	0.03	10	3.8	0.2	4	20
7	0	0	0	0.24	0.24	1640	5.7	0.78	14	70
11	0	0	0.14	0.01	0.15	7	6.0	0.18	6	30
9	0	0.07	0.33	0.05	0.44	16	9.0	0.63	13	65
14	0	13.01	0.18	10.6	23.8	169	33.0	1.08	20	100
15	0	0.06	0.30	0.95	1.30	1038	40.0	0.8	19	95
16	0.17	0	0.17	0.6	0.95	478	89.3	0.8	17	85
13	0	1.18	0.22	3.66	5.05	121	175	1	19	95
17	0	0	6.84	38.4	45.3	967	323	1.49	20	100
18	0.32	1.71	0.15	21.9	24.1	739	432	1.34	20	100
19	0	0.16	0.09	8.92	9.17	922	444	1.18	20	100
21	0	0.28	13.7	111	125	16	934	1.6	20	100
20	1.64	30.1	6.79	111	150	35	1055	1.39	20	100
22	0	24.7	0.15	69.8	94.6	813	1270	1.34	20	100

[a] Gloves assorted according to increasing total allergen content (AU/ml). Detection limits for allergens: Hev b 1, 50 ng/g; Hev b 3, 50 ng/g; Hev b 5, 7.5 ng/g; Hev b 6.02, 7.5 ng/g. Values below these limits are denoted by zero.
[b] μg/g Glove.
[c] Allergen units by IgE-ELISA inhibition (AU)/ml (1:5, w/v extract).
[d] Median wheal diameter (mm) produced by glove extract in 20 latex allergic patients:diameter (mm) produced by histamine.

b 3 are highly significant allergens for NRL-allergic children with spina bifida or other congenital malformations requiring multiple surgical operations at an early age. A quantitative capture-ELISA for the measurement of these four NRL allergens (FITkit) has recently been developed as a collaborative effort between a group of researchers and a Finnish biotechnology company (FITBiotech, Tampere, Finland). The test uses specific monoclonal antibodies against the four allergens and either purified allergens or proteins produced by recombinant DNA technology as standards. According to the manufacturer's information (FITkit insert leaflets, www.fitbiotech.com), the limits of detection for the four allergens range from 0.1 μg/liter (Hev b 6.02) to 2.3 μg/liter (Hev b 3). Repeatability ranges from 2.8 to 5.8%, and reproducibility from 2.6 to 7.6%.

2.2.2.2. Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02 in 22 NRL gloves collected in 1995. Extracts of a series of 22 NRL gloves (20 medical and 2 household gloves), marketed in Finland and worldwide in 1995, were obtained from a nationwide market survey carried out in Finland by the National Agency of Medicines in 1994–1995. Extracts (1:5, w/v) that had been stored at −20 °C were thawed, reanalyzed by human IgE-ELISA assay for total NRL allergen content, and analyzed with the FITkit according to the manufacturer's instructions (FITkit insert leaflets, www.fitbiotech.com). The contents of the four allergens were compared with the total allergen activity and SPT reactivity in 20 NRL-allergic volunteers. A preliminary report based on this glove series was recently published in abstract form [16]. The principal findings are compiled in Table 1. It can be seen that Hev b 6.02 was measurable in 18 of 22 gloves, Hev b 5 in 16, Hev b 3 in 10, and Hev b 1 in 3 glove extracts. Hev b 6.02 and Hev b 5 were present in virtually all NRL glove extracts in which specific NRL allergen activity could be demonstrated with human IgE-based assays. Hev b 3 and Hev b 1 were present less often (not measurable in 10 of 12 low-allergen gloves), but still frequently detectable in high-allergen gloves (either Hev b 1 or Hev b 3 or both were detectable in 9 of 10 of the moderate-high-allergen gloves).

Hev b 1 concentrations were the lowest of the four allergens, ranging from <0.05 to 1.64 μg/g. Hev b 3 ranged from <0.05 to 30.05 μg/g, Hev b 5 from <0.0075 to 13.67 μg/g, and Hev b 6.02 from <0.0075 to 111.3 μg/g. The sum of the quantities of the four allergens in each glove (μg/g) correlated highly significantly with the total allergen activity ($r = 0.90$) and with SPT ($r = 0.95$) (Fig. 1), but not with total protein measured by the modified Lowry method ($r = -0.11$).

Three of the twenty-two gloves (14%) did not contain measurable amounts of any of these four allergens. Hev b 6.02 was found as the only allergen in three and Hev b 5 alone in two gloves, whereas Hev b 3 and Hev b 1 were always accompanied by one to three other allergens. Similar patterns of distribution have been observed in our further studies in 1999–2001 although the total number of high-allergen gloves has considerably decreased and the presence of Hev b 1 has turned out to be very rare.

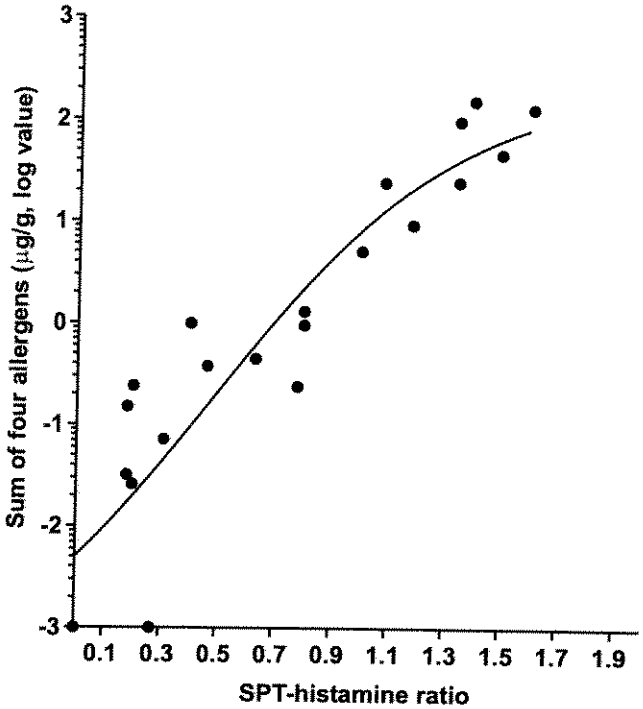

Fig. 1. Correlation between the sum of four NRL allergens (Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02), expressed as nanograms per gram of glove extract, in 22 NRL gloves, and SPT reactivity (as SPT–histamine ratio), in 20 NRL-allergic patients ($r = 0.95$; Spearman's correlation coefficient). SPT–histamine ratio refers to the median wheal diameter (mm) produced by glove extract divided by diameter of the wheal produced by histamine (10 mg/ml). For graphic purposes, gloves that did not contain measurable amounts of any of the four allergens were given an arbitrary allergen value of 10 ng/g.

2.2.2.3. Correlation between specific allergen content and SPT reactivity. As seen in Fig 1, the quantitative sum of the four allergens (Hev b 1, 3, 5, and 6.02) in the 22 gloves correlated in a highly significant manner with the SPT reactivity of 20 NRL-allergic patients. Seven of twelve low-allergen gloves (<10 AU/ml) contained small amounts of one or two of the four allergens and some patients (median 5) reacted against them in SPTs (Table 1). Yet, due to the common presence of several allergens at the same time in gloves it is still premature to suggest safety levels for each individual allergen. One may see though that the majority of the 20 NRL-allergic patients showed positive SPT reactions to gloves when the sum of the four allergens analyzed exceeded approximately 1.0 μg/g, or at the level of individual allergens, when the concentration of Hev b 1 or Hev b 3 reached or exceeded about 0.25–0.30 μg/g, Hev b 5 about 0.20–0.25 μg/g, and Hev b 6.02 about 1.0 μg/g.

Table 2
NRL allergens in 58 gloves collected in Finland in 1999[a]

Glove code	Type	AU/ml	Hev b 1 (μg/g)	Hev b 3 (μg/g)	Hev b 5 (μg/g)	Hev b 6.02 (μg/g)	Sum of specific protein (μg/g)
32	E	0.2	0	0	0	0	0
3	S	0.3	0	0	0	0	0
40	E	0.4	0	0	0	0	0
49	E	0.5	0	0	0	0	0
38	E	0.5	0	0	0	0	0
11	S	0.5	0	0	0	0.026	0.026
8	S	0.6	0	0	0	0	0
24	S	0.6	0	0	0	0	0
34	E	0.7	0	0	0	0	0
9	S	0.7	0	0	0	0	0
4	E	0.8	0	0	0	0	0
7	S	0.8	0	0.36	0	0	0.36
17	E	0.9	0	0	0	0	0
31	E	0.9	0	0	0	0	0
45	E	1	0	0	0	0	0
58	E	1	0	0	0	0	0
6	S	1	0	0	0	0.08	0.08
14	S	1	0	0	0	0	0
5	S	2	0	0	0	0	0
53	E	2	0	0	0	0	0
44	E	2	0	0	0	0	0
18	S	2	0	0	0	0	0
35	E	3	0	0	0	0.03	0.03
37	E	3	0	0	0	0	0
23	S	4	0	0	0	0	0
39	E	5	0	0	0	0	0
55	E	5	0	0	0	0.11	0.11
26	E	5	0	0	0	0.04	0.04
46	E	5	0	0	0	0	0
57	E	5	0	0	0	0	0
41	E	6	0	0	0	0.08	0.08
29	E	8	0	0	0.03	0	0.03
42	E	9	0	0	0	0.43	0.43
22	S	9	0	0	0	0	0
12	S	9	0	0	0.02	0.10	0.11
30	E	9	0	0	0.04	0	0.04
16	S	10	0	0.09	0	0.20	0.29
25	E	10	0	0	0.01	0.04	0.05
36	E	11	0	0.22	0	0.16	0.38
33	E	13	0.09	0.55	0	0.08	0.72
28	E	14	0	0	0.06	0.05	0.10
47	E	14	0	0.15	0.03	0.03	0.21
50	E	16	0	0	0.05	0.09	0.13
15	S	16	0	0.05	0	1.08	1.13
20	S	17	0	0.07	0.03	0.12	0.21
2	S	19	0	0.28	0.02	0.06	0.36
48	E	19	0	0	0.06	0.91	0.97
56	E	20	0	0	0.17	2.86	3.03
13	S	20	0	0.31	0.05	0.11	0.46
43	E	28	0	0.77	0.09	0.52	1.38
54	E	42	0	0.94	0.08	0.79	1.80
27	E	69	0	0	0.34	3.51	3.84
21	S	70	0	0	0	0.21	0.21
51	E	114	0	0	0.46	1.28	1.74
19	S	142	0	0.06	0.49	0.94	1.48
52	E	142	0	0	1.34	6.22	7.56
1	S	160	0	5	0.47	1.64	7.11
10	E	295	0	0.25	0.94	3.62	4.80

[a] Gloves are assorted according to their total allergen content (AU/ml). E, Examination glove, S, surgical glove. Detection limits for allergens: Hev b 1, 50 ng/g; Hev b 3, 50 ng/g; Hev b 5, 7.5 ng/g; Hev b 6.02, 7.5 ng/g. Values below these limits are denoted by zero.

2.2.2.4. Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02 in 58 medical gloves collected in 1999. A second series, comprising 58 medical gloves (21 surgical and 37 examination gloves), marketed in Finland and worldwide in 1999, has subsequently been analyzed for total latex allergen content in a nationwide screening (Natural Rubber Content of Latex Gloves—A Market Surveillance Study, TLT-INFO 1/1999, www.nam.fi). With respect to total allergen content, 5 had high allergen content (>100 AU/ml, range 114–295 AU/ml), 17 tested moderate (range 10–270 AU/ml), 22 tested low (1–9 AU/ml), and 14 as negligible (<1 AU/ml). Occurrence of the four NRL allergens in these gloves in relation to their total allergen content is summarized in Table 2. Also in this series, the sum of the four allergens (μg/g glove) showed a highly significant correlation to the total allergen (AU/ml) value ($r = 0.84$). It can be seen that 25 of 58 (43%) gloves did not contain measurable amounts of any of these four allergens whereas 11 gloves in the two lowest allergen categories contained minute amounts of Hev b 3, Hev b 5, or Hev b 6.02. Compared with the data for the 1995 gloves, a remarkable overall decrease in allergen content has taken place.

The results of the two glove surveys showed that virtually all NRL gloves containing significant amounts of latex allergens measured by a human IgE-based ELISA (>10 AU/ml) contained both Hev b 6.02 and Hev b 5. It is likely that Hev b 6.02 and Hev b 5 are responsible for a majority of latex allergen levels in currently marketed medical gloves. The occurrence of Hev b 3 seems to be on the decrease and Hev b 1 was seen as a rarity in gloves currently marketed in Finland. Yet, unpublished observations by our group and others confirm that especially Hev b 3 continues to be detectable in NRL medical gloves sold elsewhere and the same holds true also for Hev b 1. Finally, the novel capture-ELISA provides an easy tool for accurately quantifying NRL allergens in various materials and by means of SPT in a representative patient material it will become possible to set up recommendations for acceptable threshold levels.

3. Concluding remarks

Minimizing allergen concentration in latex goods to prevent sensitization to NRL and thereby the development clinical allergy is acknowledged as a mutual interest for rubber manufacturers and regulatory health authorities. The Food and Drug Administration (FDA) in the United States and the European Committee for Standardisation (CEN) have acknowledged the measurement of total protein as a simple option for glove manufacturers to monitor their products. However, measuring total protein, instead of specific allergens, cannot be deemed a satisfactory regulatory activity to control allergen content of rubber products. On the other hand, recent research has brought specific methods for measuring NRL allergen levels in the gloves, and this progress has already led some health or regulatory authorities to inform consumers about the highly allergenic glove brands in the market (information available, e.g., from www.nam.fi). However, the early methods are based on human IgE-containing reagents that lack standardization and are not readily available.

Currently, several relevant NRL allergens can be measured by capture-ELISA-based assays using monoclonal antibodies and purified or recombinant allergens. Such methods are specific, they can be properly standardized, and they are of sufficient sensitivity and reproducibility. Further research seems to be warranted to finally assess and reassert what combination of NRL allergens is required to cover all eventualities in NRL products. For example, it should be kept in mind that, besides hevein (Hev b 6.02), little is known about the molecular forms and epitope specificities of proteins and peptides that can resist the harsh rubber manufacturing process and retain their allergen nature. However, given the evidence available today, it seems that measuring the four clinically relevant NRL allergens, i.e., Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02, each of which has unequivocally shown to be present in some glove extracts, provides very reliable information on the allergenic properties of NRL gloves. A highly significant correlation emerged when the sum quantity of these allergens was related to results from human IgE-based assays, such as SPT and IgE ELISA inhibition. The obvious question of whether further allergens need to be added to the currently available framework should be solved after extended research. With these new methods, the assessment of threshold levels for safety purposes, which could eventually become guidelines for the rubber industry and regulatory health authorities, is becoming possible. This information would be clearly more specific and accurate than that available from total protein measurement. Eventually, this progress is expected to lead to a declining incidence of latex allergy.

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